

Floral Dimorphism in *Psychotria boninensis* Nakai (Rubiaceae) Endemic to the Bonin (Ogasawara) Islands

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(Received on March 27, 2007)

Floral and reproductive characters were examined to determine mode of dimorphism in the flowers of *Psychotria boninensis* Nakai, a species endemic to the Bonin (Ogasawara) Islands, Japan. The flowers reciprocally differed in stigma and anther height, and were composed of long- and short-styled morphs. Pollen grains from the short-styled morph were larger than those from the long-styled morph in size, but no major difference was found between the two morphs in exine sculpture. The short-styled flowers produced less pollen grain than the long-styled flowers, as in some other typically distylous species. The pollen grains produced by the two morphs were positively stained with Cotton Blue solution, and ovaries of the two morphs produced fruits and seeds in field. These results suggest that the flowers of *P. boninensis* are morphologically distylous, as in most of the typically distylous species, and that the long- and short-styled flowers are functionally bisexual. This is the first record of distyly among the plants endemic to the Bonin Islands.

Key words: Bonin (Ogasawara) Islands, distyly, *Psychotria boninensis*, reciprocal herkogamy, Rubiaceae.

Psychotria L. is a large genus comprising more than 1500 species and is widespread from tropical to subtropical regions throughout the world (Hamilton 1989, Nepokroeff et al. 1999). Most of the *Psychotria* species are shrubs or small trees, and generally grow on the forest floor. Their whitish, small flowers are usually campanulate or tubiform in shape and radially symmetrical. For the genus, the most interesting feature of the flowers is a distyly (heterostyly) that is a genetically determined reciprocal herkogamy of stigma and anther height. In distylous species the flowers are composed of long- and short-styled morphs and usually have an additional incompatibility system that prevents

self and intramorph matings (Ganders 1979, Barrett 1992), although it is known in several distylous species that their flowers show self-compatibility as well as compatibility among the intramorphs (Riveros et al. 1995, Barrett et al. 2000). In Mesoamerican *Psychotria*, it is noted that of the 66 taxa 44 are morphologically distylous and the others are monomorphic in floral morphology (Hamilton 1989). Meanwhile, in Hawaiian *Psychotria*, it has been reported that all of the species (11 spp.) are morphologically distylous (Sohmer 1977, Wagner et al. 1990). For Japanese *Psychotria* species, however, available information on sexual dimorphism is absent.

Psychotria boninensis Nakai, which is a species endemic to the Bonin (Ogasawara) Islands, has been regarded as monomorphic in floral morphology (Ono and Kobayashi 1985, Yamazaki 1989, 1993, Toyoda 2003). However, in our preliminary study the species appeared to be morphologically dimorphic with a distylous nature, as in other species of the genus. We wonder whether *P. boninensis* is truly distylous in floral morphology or not. Meanwhile, in the oceanic islands such as the Hawaiian Islands and the Bonin Islands, it is pointed out that dioecism has frequently occurred in various taxa (Carlquist 1974, Baker and Cox 1984, Kawakubo 1990, Kato and Nagamasu 1995, Sakai et al. 1995, Sugawara et al. 2004). Actually, in the Hawaiian Islands all of the *Psychotria* species just mentioned above are functionally unisexual (dioeious) despite their distylous nature in floral morphology (Wagner et al. 1990, Sakai et al. 1995). In the Bonin Islands, however, it is unclear whether *P. boninensis* is functionally unisexual or not.

In this study, we investigated floral characters to determine the dimorphism in flowers of *Psychotria boninensis* endemic to the Bonin Islands and further to make clear the reproductive nature of the species.

Materials and Method

Psychotria boninensis Nakai is a terrestrial or climbing subshrub and occurs on the floor or at the margin of the evergreen broad-leaved forests in the Bonin (Ogasawara) Islands, Japan. The plants concerned usually open flowers from May to June. Their flowers are relatively small, less than 5mm long, and somewhat campanulate in shape. Each flower lasts for a single day. Fruits mature in September to October. In this study we examined 41 individual plants in total, collected from Mt. Shigure-yama (alt. ca. 200 m) and its surrounding area on Chichijima Island, the Bonin Islands.

To observe morphological differentiation, several flowers after anthesis and flower buds before anthesis were randomly collected from the plants growing in the study sites and preserved in 70% ethanol. We measured five floral characters for each plant (see Fig. 1A): corolla length, corolla tube length, stigma height, stamen height, anther length and pollen size. For measurement of pollen size, anthers within a flower bud before anthesis were crushed in Cotton Blue in lactophenole. Subsequently, the polar diameter of 50 randomly selected pollen grains was measured using a light microscope. The number of pollen grains per flower was also counted in two flowers for each plant. In this case, one of the five anthers within a flower bud before anthesis was crushed on a lattice glass slide (Matsunami Glass Ind., Ltd.) with Cotton Blue solution under micro-binocular. In addition, pollen stainability (fertility) of more than 200 grains per flower was examined using aniline blue in lactophenole. For evaluating pollen stainability two to three flowers per plant were examined. Ovary was sectioned transversely to ascertain ovules.

For scanning electron microscope (SEM) observation, flowers and their parts (anthers and stigmas) were dehydrated in an ethanol: t-butanol series. Dehydrated materials were freezing-dried using a freeze-drying device (JFD-300, JEOL), mounted onto SEM stubs on double-sided carbon tape, coated with gold using an ion sputter (JFC-1100E, JEOL) and observed using a scanning electron microscope (JSM-5600LV, JEOL).

Observations

Dimorphism in floral morphology

Flowers of *Psychotria boninensis* were radially symmetrical with a corolla. Corolla tubes were short, about 2 mm long, and their upper portion had many oblique-facing hairs on their inner surface (Figs. 1, 3).

Observations of *Psychotria boninensis* in

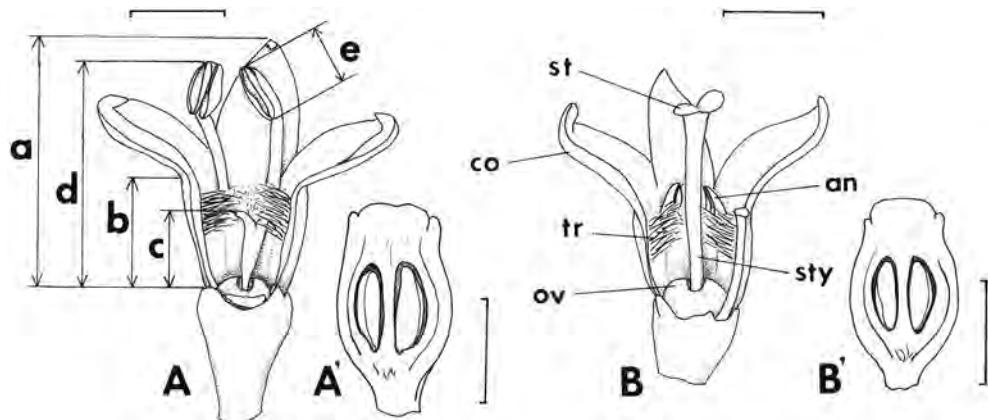


Fig. 1. Drawings of long- and short-styled morphs of *Psychotria boninensis*. A. Short-styled flower. a. Corolla length. b. Corolla-tube length. c. Stigma height. d. Anther height. e. Anther length. A'. Longitudinal section of young fruit in the short-styled flower, showing two developed seeds. B. Long-styled flower. an. Anther. co. Corolla lobe. ov. Ovary. st. Stigma. sty. Style. tr. Oblique-facing long hairs. B'. Longitudinal section of young fruit in the long-styled flower, showing two developed seeds. All scale bars = 2 mm.

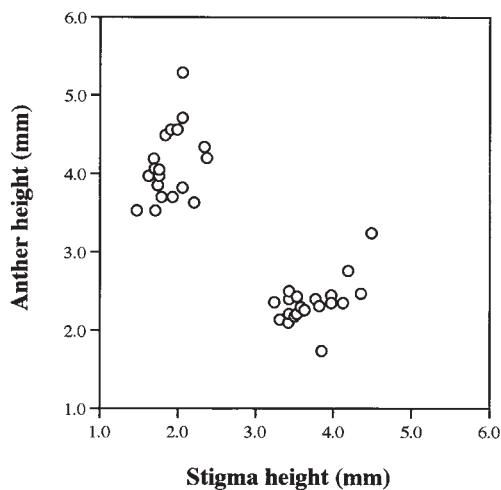


Fig. 2. Scatter diagram showing a relationship of stigma height and anther height in *Psychotria boninensis*.

a natural population revealed the presence of two distinct floral morphs: long-styled morph with short stamens and short-styled morph with long stamens (Figs. 1, 2). At the study site, 49% (20/41) of the plants sampled

were long-styled morph and 51% (21/41) short-styled one. In the long-styled morph, a style was exserted from the corolla tube and bifurcated, forming a stigma, while stamens remain within a corolla tube and their anthers, especially their lower half, were concealed with a number of long, oblique-facing hairs (Figs. 1B, 3A₁). In the short-styled morph, on the other hand, stamens with long filaments were exserted from the corolla tube and their anthers dehisced introrsely, and a style was positioned below the anthers and its stigma was wholly concealed with a number of oblique-facing hairs born on the corolla tube (Figs. 1A, 3B₁).

Between the two morphs there was no significant difference in corolla-tube length (Table 1). However, the two morphs differed significantly from each other in corolla length and anther length (Table 1).

Dissected flower buds of the two morphs were examined to show the relation of stigma height, anther height and position of long hairs attached to the corolla tube. We focused on the oblique-facing hairs borne on

Table 1. Comparison of long- and short-styled morphs in several floral and reproductive characters of *Psychotria boninensis*

Floral trait	Long-styled	Short-styled	Statistics
	Mean ± SD (N)*	Mean ± SD (N)*	
Corolla length (mm)	4.4 ± 0.4 (20)	4.7 ± 0.3 (21)	0.001 < p < 0.01
Corolla-tube length (mm)	2.2 ± 0.3 (9)	2.3 ± 0.2 (12)	ns
Stigma height (mm)	3.7 ± 0.4 (20)	1.9 ± 0.3 (21)	p < 0.001
Anther height (mm)	2.4 ± 0.3 (20)	4.2 ± 0.5 (21)	p < 0.001
Anther length (mm)	1.0 ± 0.1 (20)	1.1 ± 0.1 (21)	0.001 < p < 0.01
Diameter of pollen grain (μm)	38.1 ± 1.9 (20)	50.5 ± 2.8 (21)	p < 0.001
Pollen number per flower	4460 ± 762 (10)	2887 ± 462 (10)	p < 0.001
Stainability of pollen grain (%)	94.9 ± 5.6 (20)	93.3 ± 7.8 (21)	ns

*N represents number of individual plants examined.

**Statistics tests (Student's *t*-test) were carried out between long- and short-styled morphs.

the corolla tube. In young flower buds of the two morphs, stamen height and style height were equal to each other and the hairs were positioned below the stigma and stamens (Fig. 3A₃, B₃). In flower buds before anthesis, the oblique-facing hairs of the long-styled morph were positioned below the stamens and stigma (Fig. 3A₂), like those in the mature flowers, while the hairs of the short-styled morph concealed the stigma entirely (Fig. 3B₂).

Dimorphism in pollen size and number of pollen grains per flower

Pollen grains from the two morphs were spherical and tri-colporate in aperture (Fig. 4). Polar diameter of pollen grains from the short-styled morphs was significantly longer than those from the long-styled morphs (Student's *t*-test, p < 0.001; Fig. 5, Table 1). Exine sculpture of pollen grains from the both morphs showed complex reticulation. Between the two morphs, no major difference was found in exine sculpture (Fig. 4).

Number of pollen grains per flower was compared between the two morphs. Although the number of pollen grains per flower varied considerably within a plant or among plants, it significantly differed between the long- and the short-styled

morphs (Student's *t*-test, p < 0.001). As shown in Table 1, average number of pollen grains per flower was 4460 in the long-styled morph and 2887 in the short-styled morph, respectively, so the pollen number per flower of the long-styled morph was more than 1.5 times relative to that of the short-styled morph.

Stainability of pollen grains and fruit production

Each of the two morphs showed high pollen stainabilities. Average values were 94.9% for the long-styled morph and 93.3% for the short-styled morph, respectively (Table 1). Statistically, there was no significant difference between the two morphs in pollen stainability (Student's *t*-test, p > 0.05).

Meanwhile, flowers of the two morphs often produced fruits under natural populations, and their fruits usually contained two seeds in each ovary (Fig. 1A', B'), although the fruit and seed productions of the two morphs were not examined quantitatively in the present study. It is certain that the pistils of the two morphs are functional, since they produce fruits and seeds.

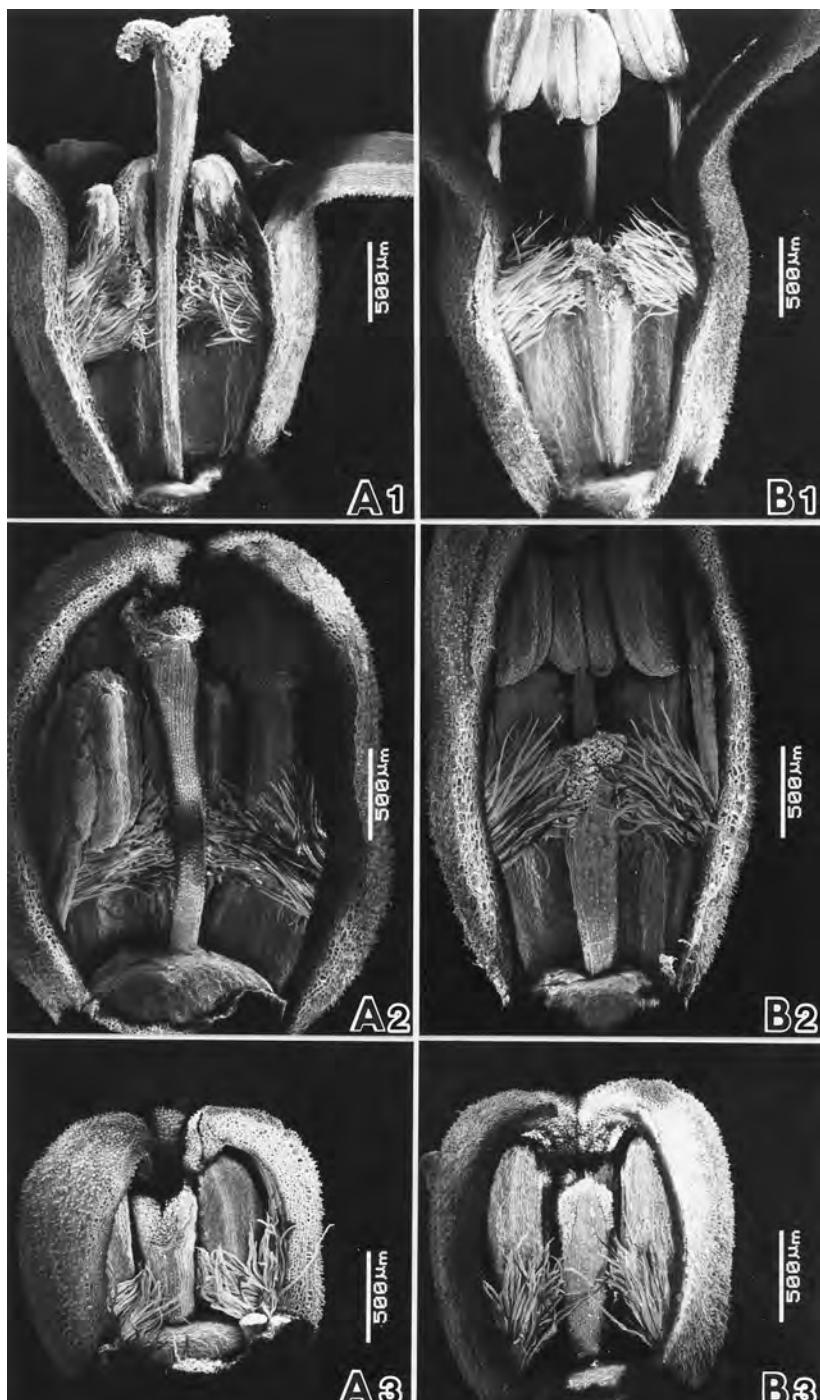


Fig. 3. SEM photographs of flowers and flower buds in long- and short-styled morphs of *Psychotria boninensis*. A₁. Long-styled flower after anthesis. A₂. Long-styled flower before anthesis. A₃. Young flower bud of long-styled morph. B₁. Short-styled flower after anthesis, in which stigma was covered with a number of oblique-facing long hairs. B₂. Short-styled flower before anthesis. B₃. Young flower bud of short-styled morph, in which stigma was exserted from the upward-facing hairs.

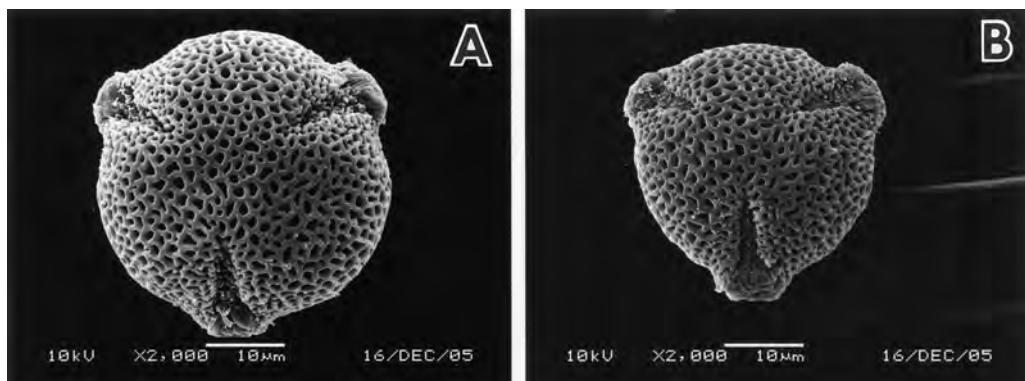


Fig. 4. SEM photographs of pollen grains in long- and short-styled morphs of *Psychotria boninensis*. A. Pollen grain from a stamen in the short-styled morph. B. Pollen grain from a stamen in the long-styled morph.

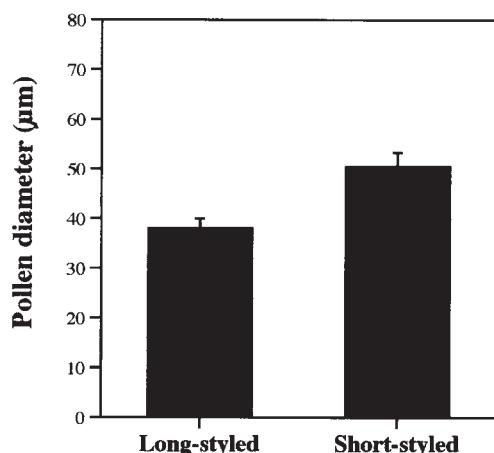


Fig. 5. Polar diameter of pollen grains from long- and short-styled morphs of *Psychotria boninensis*. Vertical lines indicate standard deviations. Sample size and statistics refer to Table 1.

Discussion

The present studies revealed that the flowers of *Psychotria boninensis* were morphologically distylous and reciprocally differed in stigma and anther height. Moreover, pollen grains from the short-styled morph were larger than those from the long-styled morph, and the short-styled flowers produced less pollen grain than the long-styled

flowers. In these respects the floral dimorphism found in *P. boninensis* is very similar to that of the typically distylous (heterostylous) species reported so far (Ganders 1979, Barrett 1992, Dulberger 1992, Sugawara et al. 2002). Therefore, *P. boninensis* endemic to the Bonin Islands may be regarded as distylous, like other species of this genus (Ganders 1979, Hamilton 1989), although there still remains uncertainty as to whether the species has an incompatibility system that prevents self and intramorph matings. This is the first record on stylar dimorphism from the plants endemic to the Bonin Islands.

In the oceanic Hawaiian Islands, it is well known that dioecy is frequent among endemic species (Carlquist 1974, Baker and Cox 1984, Sakai et al. 1995). As already stated in the introduction, all of the *Psychotria* species (11 species) within the islands are functionally unisexual (dioecious) despite their distylous nature in floral morphology (Wagner et al. 1990, Sakai et al. 1995). In the species concerned here, *P. boninensis*, the long- and short-styled flowers have fertile pollen grains and produce fruits and seeds under natural conditions. These results suggest that the reproductive

nature of *P. boninensis* is functionally bisexual, in contrast with that of the Hawaiian *Psychotria* species.

An interesting feature in the flowers of *Psychotria boninensis* is the oblique-facing long hairs found on the corolla tube of the two morphs. Contrary to that of the long-styled morphs, the stigmatic surface of the short-styled morphs is wholly concealed by a number of the long oblique-facing hairs on the corolla tube (Fig. 3). Such hairs may be common in the genus but are rarely found in the other genera of the same family (Hamilton 1989). In general, the hairs found on the corolla tube are regarded as excluding nectar or pollen robbers such as syrphids and/or as preventing evaporation of nectar from the corolla (Naiki and Kato 1999). We have no evidence for these suggestions in our study species. Meanwhile, if the corolla tube has no hairs that conceal a stigmatic surface, clogging by the self-pollen grains may occur on the stigma, especially of the short-styled morph, as noted in the flowers of *Daphne kamtschatica* var. *jezoensis* (Kikuzawa 1989). Therefore, it seems probable that the long hairs found on the corolla tube of *P. boninensis* prevent landing of the pollen grains from the same flowers in pollination system. To clarify these speculations, further studies especially on pollination systems are needed.

In conclusion, the present results indicate that the flowers of *Psychotria boninensis* endemic to the Bonin Islands are morphologically distylous, consisting of long- and short-styled morphs and reciprocally differ in stigma and anther height. It is also confirmed that both long- and short-styled flowers of *P. boninensis* are functionally bisexual.

We thank to Dr. H. Kato for his help in field survey. We are also grateful to the Environmental Agency of the Japanese Government, for permission to collect samples used in this study.

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近藤よし美^a, 西出真人^b, 渡邊謙太^b, 菅原 敬^b:
小笠原諸島固有植物オオシラタマカズラ（アカネ科）における花の二型性

小笠原諸島固有植物オオシラタマカズラ（ボチョウジ属）の花は形態的には単型であるとみなされてきた (Ono and Kobayashi 1985, Yamazaki 1989, 1993, Toyoda 2003)。ところが野外調査を通じて、この種の花は同属内に一般に知られる二型花柱性である可能性が出てきた。小笠原諸島同様に海洋島として知られるハワイ諸島では、ボチョウジ属植物が11種（いずれも固有種）分布するが、そのすべてが二型花柱性的特徴を有し、一方で機能的には单性化し雌雄異株へと進化していることが報告されている (Sohmer 1977, Wagner et al. 1990, Sakai et al. 1995)。そこで小笠原諸島のオオシラタマカズラが本当に二型花柱性を示すかどうかを確認するとともに、その性的機能についても調べた。その結果、オオシラタマカズラは二型花柱性、

すなわち長花柱型と短花柱型の花を有し、それらの花粉のサイズは短花柱型よりも長花柱型において有意に小さく、花当たりの花粉生産量も長花柱花において有意に多いという結果が得られた。これらは他の二型花柱性植物群に一般に見られる特徴ともよく一致する。一方機能的には、いずれの花型でも花粉はコットンブルー染色液に90%以上の染色性を示し、またそれぞれの花は野外で果実や種子をつくりっていた。これらの結果は、オオシラタマカズラ二型花がハワイ諸島のように機能的单性花にならず、両性花であることを示している。二型花柱性は小笠原諸島固有植物では初めての確認であるが、同属のもう一種についても同様の調査を現在進めている。

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